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AWARD NUMBER: W81XWH-14-1-0562

TITLE: Development of a Lubricant Therapy to Prevent Development of Osteoarthritis after Acute Injury of Synovial Joints

PRINCIPAL INVESTIGATOR: Robert L. Sah

RECIPIENT: Dr. Prem Yadav, Ph.D.

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14. ABSTRACT <u>Purpose.</u> The prevention of early-onset osteoarthritis after traumatic joint injury remains a clinical challenge and may be associated with the poor lubricant quality of the synovial fluid in the injured joint. <u>Scope.</u> The aims of the studies here were to test whether the pathological accumulation of a specific substance found in joint fluid following an injury mediated altered synovial fluid lubrication of articular cartilage and whether modulation of lubricating molecules could restore lubrication function and prevent cartilage wear. <u>Major Findings.</u> Through studies in year 1, we have delineated the time-dependent changes in lubricant molecules and function of joint fluid post-injury in a rabbit model as well as early, mid, and late changes post-injury in human synovial fluid. <u>Progress and Significance.</u> Collection and analysis of human synovial fluid is ongoing (Aim 1a), and we have completed most of the analysis of rabbit synovial fluid (Aim 1b). From this data, the time-dependent relationship between joint fluid molecules and function could be determined.					
15. SUBJECT TERMS Cartilage, Wear, Lubrication, Friction, Post-traumatic osteoarthritis, Injury, Blood, Synovial Fluid, Joint					
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**1. INTRODUCTION:** Deficient lubrication of articular cartilage by synovial fluid after synovial joint injury may advance the deterioration of cartilage, especially in the superficial zone, and hasten the development of osteoarthritis (OA). We recently delineated two mechanisms by which acute injury disrupts the normal lubrication of articular cartilage by synovial fluid: (1) diminished concentration of high-MW HA in SF, and (2) detrimental interaction of blood with SF. The scope of these studies was to test whether the impaired lubrication of articular cartilage by synovial fluid after joint injury contributes to the development of post-traumatic OA and is due, in part, to the pathological accumulation of a specific substance which can be countered by an enhanced lubricant therapy.

**2. KEYWORDS:** cartilage wear, lubrication, friction, synovial fluid, joint, osteoarthritis, post-traumatic osteoarthritis, injury, blood.

**3. ACCOMPLISHMENTS:**

**3.a. What were the major goals of the project?**

Specific Aim 1 (per proposal)	Timeline	Site 1
<b>1.1. From 60 patients, collect hSF &amp; analyze for lubricant content, quality, &amp; function.</b>	Months	Completion [%]
(a) Obtain IRB approval (VA Palo Alto).	1-3	100
(b) Collect hSF, & transport to UCSD.	3-24	50
(c) Analyze hSF (as fluid is collected) for biochemical properties.	3-30	50
(d) Analyze hSF for lubricant function.	3-30	50
<b><i>Milestone(s) Achieved:</i></b>		
A. VA Palo Alto IRB Approval	3	100
B. Characterization of hSF with time after injury and severity/type of injury	15	75
<b>1.2. After rabbit ACL Transection (ACLT), collect &amp; analyze (1) rSF for lubricant content, quality, &amp; function and (2) rAC for content and structure.</b>	Months	Completion [%]
(a) Obtain IACUC approval (UCSD)	1-3	100
(b) Perform rabbit ACLT surgeries, collect fluids & articular cartilage.	3-4.5	100
(c) Analyze rSF for biochemical properties.	3-6	67
(d) Analyze rSF for lubricant function.	3-6	33
(e) Analyze rAC for biochemical and molecular properties.	3-9	75
<b><i>Milestone(s) Achieved:</i></b>		
(A) UCSD IACUC Approval	3	100
(B) Characterization of rSF and rAC with time after injury (rabbit ACLT)	9	50

**3.b. What was accomplished under these goals?**

3.b.i. Aim 1a.

Major Activities. IRB approval was obtained at VA Palo Alto (IRB#33169) and UCSD (IRB#141410X), and the study was approved by the HRPO (A18193). hSF samples were collected at time points early (A1a, 0-14days, n=4), mid (A1b, 15-30days, n=4), and late (A1c, 31-90days, n=12) post-injury. They were analyzed for the concentrations of lubricant molecules, proteoglycan-4 (PRG4) and hyaluronan (HA), and lubricant function. Values obtained for injury samples were compared to historical normal hSF (NL-hSF), SF from knees with a chronic meniscus tear (MEN) and from osteoarthritic knees (OA-hSF).

Specific Objectives. The objectives for Year 1 were to a) obtain IRB approval of the study, b) collect human synovial fluid (hSF) and transport to UCSD, c) analyze hSF for biochemical properties (as it is collected), and d) analyze hSF for lubricant function.

Significant Results, Key Outcomes, Major Findings, Developments, or Conclusions. Lubricant molecule concentrations and quality as well as lubricant function were modulated by injury relative to NL samples. Relative to NL samples, protein concentrations were higher (+115-157%) in injury samples. Similarly, PRG4 concentrations were higher (+57-87%) in injury than NL samples. Relative to NL samples, HA concentrations were lower (-69-89%) in injury samples, with a shift in HA molecular weight (MW) to lower MW forms in injury samples. GAG concentrations were similar between injury groups. Generally, the kinetic ( $\mu_{kinetic}$ ) and start-up ( $\mu_{static, Tps=120}$ ) friction coefficients were higher in A1a than NL samples (+23-25%).

The concentrations and quality of SF lubricant constituents were altered following injuries involving the ACL and/or meniscus, in a manner consistent with our previous studies. SF samples will be analyzed for the suspected pathological substance as well as glycosaminoglycan (GAG) and collagen content to assess the extent of active cartilage degradation. It would be of interest to assess these properties in additional samples to better define the time-dependent changes in human SF at early time points following acute joint injury.

3.b.ii. Aim 1b.

Major Activities. IACUC approval was obtained at UCSD (IACUC Protocol #S10018). Unilateral anterior cruciate ligament transection (ACLT) surgeries in the rabbit were performed to define the time-dependent changes in synovial fluid (rSF) and articular cartilage (rAC, Aim 1b). Analysis of rSF and rAC for iron and iron-binding proteins, biochemical content, and lubricant function are on-going.

Specific Objectives. The objectives for Year 1 were to a) obtain IACUC approval of the study, b) perform rabbit ACLT surgeries, collect fluids & articular cartilage (for Aim 1b), c) analyze rSF for biochemical properties, d) analyze rSF for lubricant function, and e) analyze rAC for biochemical properties.

Significant Results, Key Outcomes, Major Findings, Developments, or Conclusions. Lubricant molecule concentrations and quality were modulated by injury relative to

contralateral control (CTRL) samples. Relative to CTRL samples, ACLT rSF protein concentrations were generally higher at days 1, 4, and 7 (+38-72%) and similar at days 14, 28, and 42. Relative to CTRL samples, rSF HA concentrations tended to be lower in ACLT samples at days 1 (-73%), 4 (-69%), 7 (-52%), 14 (-62%), and 28 (-50%) and similar at day 42, with HA MW being shifted to lower MW forms in ACLT samples.

The concentrations and quality of SF lubricant constituents were altered following injury involving the ACL, in a manner consistent with our previous studies. SF samples will be analyzed for the suspected pathological substance as well as lubrication function.

**3.c. What opportunities for training and professional development has the project provided?**

Trained undergraduate and graduate students in biochemical techniques and project management skills.

**3.d. How were the results disseminated to communities of interest?**

Nothing to report.

**3.e. What do you plan to do during the next reporting period to accomplish the goals?**

In the next reporting period, it is planned to complete on-going studies for both Aim 1a and Aim 1b. For Aim 1a, we plan to continue analyzing hSF as the samples arrive to UCSD. In addition, we plan to analyze the hSF for iron and iron-binding proteins. We are aiming for a publication by June 2016 of hSF biochemical and lubricating properties following injury and the relationship between iron and lubrication function of hSF.

For Aim 1b, we plan to measure iron and iron-binding proteins in the rSF and rAC, we plan to complete the lubricant analyses in rSF, and we plan to assess the rSF for lubrication function. In the first quarter of next year, we plan to begin the second rabbit study (Aim 2b) to assess the ability of a chelator to restore lubrication function in the joint. We are targeting a publication of the time-dependent, post-injury biochemical and lubrication properties of rSF for March 2016.

**4. IMPACT:**

**4.a. What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report. However, a publication is pending.

**4.b. What was the impact on other disciplines?**

Nothing to report. However, a publication is pending.

**4.c. What was the impact on technology transfer?**

Nothing to report.

**4.d. What was the impact on society beyond science and technology?**

Nothing to report. However, a publication is pending.

**5. CHANGES/PROBLEMS:**

**5.a. Changes in approach and reasons for change.**

Nothing to report.

**5.b. Actual or anticipated problems or delays and actions or plans to resolve them.**

Analyses of rabbit synovial fluids required some scaling of the various assays. That work has been completed and analyses are proceeding.

**5.c.Changes that had a significant impact on expenditures.**

Nothing to report.

**5.d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.**

Nothing to report.

5.d.i. Significant changes in use or care of human subjects

5.d.ii. Significant changes in use or care of vertebrate animals.

5.d.iii. Significant changes in use of biohazards and/or select agents

**6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:**

**6.a. Lay Press:**

**6.b. Peer-Reviewed Scientific Journals:**

**6.c. Invited Articles:**

**6.d. Abstracts:**

**6.e. Presentations:**

**7. INVENTIONS, PATENTS AND LICENSES:**

Nothing to report.

**8. REPORTABLE OUTCOMES:**

Nothing to report.

**9. OTHER ACHIEVEMENTS:**

Nothing to report.

**10. REFERENCES:**

**11. APPENDICES:**



# Development of a Lubricant Therapy to Prevent Osteoarthritis after Acute Injury

Log Number: W81XWH-14-1-0562

Award Number: OR130385



PI: Robert L. Sah

Org: University of California, San Diego

Award Amount: \$1,128,083

## Study/Aim(s)

- Determine time course & extent of increased iron in SF and articular cartilage after knee joint injury
- Optimize the dosage & time course of chelation therapy to deplete pathological iron from cartilage
- Assess the ability of chelation-enhanced lubricant therapy to restore lubrication function and prevent wear

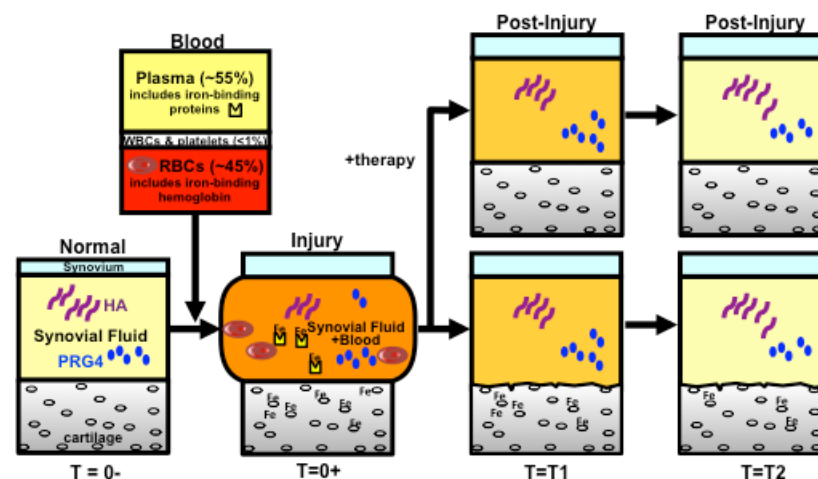
## Approach

SF will be obtained from the injured (AI) or normal (NL) joint of patients or rabbits at multiple times after injury

SF will be analyzed for iron, iron-binding proteins, and lubricant content and function.

Iron-laden cartilage following chelator treatment will be analyzed for iron content & lubrication properties.

AI-SF ±chelator ±lubricants will be assessed for wear protective properties relative to NL-SF *in vitro* and *in vivo*.



Accomplishment: Studies are in progress to analyze the SF lubricant properties after injury *in vitro* and *in vivo*. The overall goal is to develop an enhanced lubricant therapy to restore SF lubricant function after acute joint injury and prevent post-traumatic osteoarthritis.

## Timeline and Cost

Activities	CY	14	15	16	17
Obtain human & animal protocol approvals					
Determine iron profile after injury					
Optimization of chelation therapy					
Determine SF ±therapy function					
Estimated Budget (\$K)		\$95	\$378	\$375	\$282

Updated: 01/06/2015

## Goals/Milestones

**CY14 Goals** – Obtain study approvals

☒ Obtain UCSD IRB & USAMRMC HRPO approval

☒ Obtain IACUC & USAMRMC ACURO approval

**CY15 Goal** – SF characterization & Optimization of chelation therapy

☐ Collect & analyze human SF biochemical properties & function

☐ Collect & analyze rabbit SF biochemical properties & function

☐ Determine chelator dose-dependent iron removal from human cartilage

☐ Determine post-chelator hSF lubricant function

☐ Determine chelator dose-dependent iron removal from rabbit cartilage

☐ Determine post-chelator rSF lubricant function

**CY16 Goal** – Develop enhanced lubricant therapy

☐ Determine enhanced AI-hSF & AI-rSF lubricant function with chelator and lubricant supplements

## Comments/Challenges/Issues/Concerns

- None

## Budget Expenditure to Date

Projected Expenditure: \$379,938

Actual Expenditure: \$320,502